PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: John P. White
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

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Applicant's or agent's file reference 76315-A-PCT/JPW/YC	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 07/13559	07 June 2007 (07.06.2007)	07 June 2006 (07.06.2006)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68; C12M 1/34; G01N 33/48; G01N 33/50 (2007.01)
USPC - 435/6, 435/287.2, 702/20

Applicant The Trustees of Columbia University in the City of New York

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	26 September 2007 (26.09.2007)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

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Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-12, 17	YES
	Claims	13-16	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-17	NO
Industrial applicability (IA)	Claims	1-17	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 13-16 lack novelty under PCT article 33(2) as being anticipated by US 2003/0064360 A1 to Gold et al. (hereinafter "Gold").

Regarding claim 13, directed to a nucleotide having an azido group covalently bound to its base, Gold teaches nucleotide bases covalently modified with azido groups (para [0020])

Regarding claim 14, directed to the nucleotide of claim 13, wherein the nucleotide is dUTP and the azido group is bound to the base at the 5-position, Gold teaches the compound, 5-azidouracil (para [0020]) and the presence of UTP nucleotides in DNA (para [0020]).

Regarding claim 15, directed to the nucleotide of claim 13, wherein the nucleotide is dATP and the azido group is bound to the base at the 8-position, Gold teaches the compound, 8-azidoadenine (para [0020]) and the dATP nucleotide (para [0145]).

Regarding claim 16, directed to the nucleotide of claim 13, wherein the nucleotide is dGTP and the azido group is bound to the base at the 8-position, Gold teaches the compound, 8-azidoguanine (para [0020]) and the dGTP nucleotide (para [0145]).

Claims 1-8 lack an inventive step under PCT article 33(3) as being obvious over Gold, as above, in view of US 2006/0105461 A1 to Tom-Moy et al. (hereinafter "Tom-Moy")

Regarding claim 1, directed to a method for determining the nucleotide sequence of a single-stranded DNA comprising the steps of:
(a) passing the single-stranded DNA through a pore of suitable diameter by applying an electric field to the DNA, wherein at least each A or each G residue and at least each C, each T or each U residue comprises a modifying group bound to its respective base so that each type of nucleotide in the DNA has an electronic signature which is distinguishable from the electronic signature of each other type of nucleotide in the DNA;
(b) for each nucleotide of the DNA which passes through the pore, determining an electronic signature for such nucleotide; and
(c) comparing each electronic signature determined in step (b) with electronic signatures corresponding to each of A, G/C and T modified as per the nucleotides in the single-stranded DNA, so as to determine the identity of each such nucleotide, thereby determining the nucleotide sequence of the single-stranded DNA.

Gold teaches that any of the DNA nucleotide bases (A,C, G or T) may be covalently modified such as to form azido adducts (para [0020]). Gold does not teach sequencing ssDNA using a nanopore in an electric field to produce a unique electronic signature from which the nucleotide sequence may be determined.

Tom-Moy teaches sequencing (para [0033]) of single-stranded DNA (ssDNA) (para [0027]) using passage of a polynucleotide (para [0001]) through a nanopore (para [0032]) residing in an electric field (para [0001]). Tom-Moy also teaches that the polynucleotide bases may be modified (para [0027]). Tom-Moy teaches that the resultant identity and sequence of the nucleotides may be determined by their unique electronic signature (para [0031], [0033]). It would have been obvious to one of skill in the art to combine the teachings of Gold and Tom-Moy to sequence ssDNA using a nanopore and an electronic signature since the azido or other modifications of the bases as taught by Gold could be used to optimize the uniqueness in the electronic signature of individual nucleotides in the technique taught by Tom-Moy to improve sequence identification.

Regarding claim 2, directed to a method for determining the nucleotide sequence of a single-stranded RNA comprising the steps of:
(a) passing the single-stranded RNA through a pore of suitable diameter by applying an electric field to the RNA, wherein at least each A or each G residue and at least each C or each U residue comprises a modifying group bound to its respective base so that each type of nucleotide in the RNA has an electronic signature which is distinguishable from the electronic signature of each other type of nucleotide in the RNA;
(b) for each nucleotide of the RNA which passes through the pore, determining an electronic signature for such nucleotide; and
(c) comparing each electronic signature determined in step (b) with electronic signatures corresponding to each of A, G/C and U modified as per the nucleotides in the single-stranded RNA, so as to determine the identity of each such nucleotide, thereby determining the nucleotide sequence of the single-stranded RNA.

Gold teaches that any of the RNA nucleotide bases (A,C, G or U) may be covalently modified such as to form azido adducts (para [0020]). Gold does not teach sequencing ssRNA using a nanopore inan electric field to produce a unique electronic signature from which the nucleotide sequence identification.

SEE CONTINUATION SHEET

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Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing

      ☐ contained in the international application as filed

      ☐ filed together with the international application in electronic form

      ☐ furnished subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V(2):

Tom-Moy teaches sequencing (para [0033]) of single-stranded RNA (ssRNA) (para [0027]) using passage of a polynucleotide (para [0001]) through a nanopore (para [0032]) residing in an electric field (para [0001]). Tom-Moy also teaches that the polynucleotide bases may be modified (para [0027]). Tom-Moy teaches that the resultant identity and sequence of the nucleotides may be determined by their unique electronic signature (para [0031], [0033]). It would have been obvious to one of skill in the art to combine the teachings of Gold and Tom-Moy to sequence ssRNA using a nanopore and an electronic signature since the azido or other modifications of the bases as taught by Gold could be used to optimize the uniqueness in the electronic signature of individual nucleotides in the technique taught by Tom-Moy to improve sequence identification.

Regarding claims 3-5, directed to the method of claim 1 or 2, wherein the pore has a diameter of from about 1 nm to about 5 nm (claim 3), the method of claim 1 or 2, wherein the pore has a diameter of from about 1 nm to about 3 nm (claim 4) and the method of claim 1 or 2, wherein the pore has a diameter of about 1 nm, 2 nm, 3 nm, 4 nm or 5 nm (claim 5), Tom-Moy teaches nanopore diameters between 1-10 nanometers (para [0048]).

Regarding claim 6, directed to the method of claim 1, wherein each A and each T or each U residue comprises a modifying group, Gold teaches that the polynucleotide bases may be modified (para [0020]) in any of the four normal bases, A, C, G or T (para [0020]).

Regarding claim 7, directed to the method of claim 2, wherein each A and each U residue comprises a modifying group, Gold teaches that the polynucleotide bases may be modified (para [0020]) in any of the four normal bases, A, C, G or U (para [0020]).

Regarding claim 8, directed to the method of claim 1 or 2, wherein each G and each C residue comprises a modifying group, Gold teaches that the polynucleotide bases may be modified (para [0020]) in any of the four normal bases, A, C, G or T (para [0020]).

Claims 9 lacks an inventive step under PCT article 33(3) as being obvious over Gold in view of Tom-Moy, as above, and further in view of US 2005/0239194 A1 to Gorenstein et al. (hereinafter "Gorenstein").

Regarding claim 9, directed to the method of claim 1, wherein the single-stranded DNA is obtained by
(a) synthesizing double-stranded DNA using a single-stranded template, a DNA polymerase and nucleotides, wherein at least each A or each G residue and at least each C or each T residue comprises a modifying group bound to its respective base so that each type of nucleotide in the DNA has an electronic signature which is distinguishable from the electronic signature of each other type nucleotide in the DNA, and
(b) removing from the resulting double-stranded DNA the single-stranded DNA containing modified nucleotides,

Gold teaches that any of the DNA nucleotide bases (A,C, G or T) may be covalently modified such as to form azido adducts (para [0020]). Tom-Moy teaches that the resultant identity and sequence of the nucleotides may be determined by their unique electronic signature (para [0031], [0033]). Neither Gold nor Tom-Moy teaches the process of dsDNA synthesis on an ssDNA template using a polymerase and removing the dsDNA from the ssDNA containing modified nucleotides.

Gorenstein teaches the synthesis of dsDNA (para [0093]) from an ssDNA template (para [0074], [0092], [0093]) utilizing a polymerase (para [0053]) and modified nucleotides (para [0002]) as well as removing and separating resulting dsDNA from ssDNA following synthesis (para [0094]). It would have been obvious to one of skill in the art to combine the teachings of Gold, Tom-Moy and Gorenstein to prepare and identify ssDNA by the claimed method because Gorenstein's teaching of a synthetic process for ssDNA using modified nucleotides could utilize Gold's modified nucleotide bases and Tom-Moy's sequencing method would be expected to provide a method for sequencing the resulting ssDNA.

Claims 10 lacks an inventive step under PCT article 33(3) as being obvious over Gold in view of Tom-Moy, as above, and further in view of US 2004/0166282 A1 to Brown et al. (hereinafter "Brown").

Regarding claim 10, directed to the method of claim 2, wherein the single-stranded RNAis obtained by
(a) synthesizing double-stranded RNA using a single-stranded template, an RNA polymerase and nucleotides, wherein at least each A or each G residue and at least each C or each U residue comprises a modifying group bound to its respective base so that each type of nucleotide in the RNA has an electronic signature which is distinguishable from the electronic signature of each other type nucleotide in the RNA, and
(b) removing from the resulting double-stranded RNA the single-stranded RNA containing modified nucleotides,

Gold teaches that any of the RNA nucleotide bases (A,C, G or U) may be covalently modified such as to form azido adducts (para [0020]). Tom-Moy teaches that the resultant identity and sequence of the nucleotides may be determined by their unique electronic signature (para [0031], [0033]). Neither Gold nor Tom-Moy teaches the process of dsDNA synthesis on an ssRNA template using a polymerase and removing the dsRNA from the ssRNA containing modified nucleotides.

Brown teaches the synthesis of dsRNA (para [0018]) from an ssRNA template (para [0025], [0049]) utilizing a polymerase (para [0074]) and modified nucleotides (para [0025]) as well as removing an separating resulting dsRNA from ssRNA following synthesis (para [0049]). It would have been obvious to one of skill in the art to combine the teachings of Gold, Tom-Moy and Brown to prepare and identify ssRNA by the claimed method because Brown's teaching of a synthetic process for ssRNA using modified nucleotides could utilize Gold's modified nucleotide bases and Tom-Moy's sequencing method would be expected to provide a method for sequencing the resulting ssRNA.

**********SEE CONTINUATION SHEET**********

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V(2):

Claim 17 lacks an inventive step under PCT article 33(3) as being obvious over Gold, as above, in view of US 2003/0186982 A1 to Seela et al. (hereinafter "Seela").

Regarding claim 17, Gold does not teach making a modified nucleotide comprising contacting the nucleotide of claim 13 with an alkyne containing compound under conditions permitting reaction between the azido and the alkyne groups. Seela teaches reacting azido-derivatized nucleotides (para [0115]) with alkynyl moieties to generate alkynyl derivatives (para [0026], [0104], [0717]) including aminoalkynyl groups (para [0717]). It would have been obvious to one of skill in the art to combine the teachings of Gold and Seela to generate a modified nucleotide comprising an alkyne containing compound in order to further derivatize azido-nucleotide bases.

Claim 11 lacks an inventive step under PCT article 33(3) as being obvious over Gold in view of Tom-Moy, as above, and further in view of Gorenstein and Seela.

Regarding claim 11, directed to the method of claim 1, wherein the single-stranded DNA is obtained by
(a) synthesizing double-stranded DNA using a single-stranded template, a DNA polymerase and nucleotides, wherein at least each A, each G, each C, each U or each T residue comprises an azido group bound to its base, and at least each A, each G, each C, each U and each T comprises an amino group bound to its base, whereby the azido and amino groups do not reside on the same type of base,
(b) removing from the resulting double-stranded DNA the single-stranded DNA containing the azido and amino group-containing nucleotides and
(c) reacting the resulting single-stranded DNA with a first modifying group which forms a bond with the azido group and a second modifying group which forms a bond with the amino group so as to obtain the single-stranded DNA,

Gold teaches that any of the DNA nucleotide bases (A, C, G or T) may be covalently modified such as to form azido adducts (para [0020]). Gold also teaches 2'-amino group modifications of bases (para [0007]). Gold does not require that the amino modifications reside on the same base as the azido modifications and the two modifications are taught independently (para [0007], [0020]). Gorenstein teaches the synthesis of dsDNA (para [0093]) from an ssDNA template (para [0074], [0092], [0093]) utilizing a polymerase (para [0053]) and modified nucleotides (para [0002]) as well as removing and separating resulting dsDNA from ssDNA following synthesis (para [0094]). Neither Gold, Tom-Moy nor Gorenstein teach reacting the resulting single-stranded DNA with a first modifying group which forms a bond with the azido group and a second modifying group which forms a bond with the amino group so as to obtain the single-stranded DNA.

Seela teaches further modification of azido-derivatized nucleotides (para [0115]) and with alkynyl functional groups (para [0026], [0104]) to form aminoalkynyl adducts (para [0717]). It would have been obvious to one of skill in the art to combine the teachings of Gold, Tom-Moy, Gorenstein and Seela to synthesize and sequence ssDNA by the claimed method because Seela's teaching of further derivatization of the amino functional group on nucleotide bases would be expected to add an additional level of uniqueness to the DNA for improved sequencing and characterization.

Claim 12 lacks an inventive step under PCT article 33(3) as being obvious over Gold in view of Tom-Moy, as above, and further in view of Brown and Seela.

Regarding claim 12, directed to the method of claim 2, wherein the single-stranded RNA is obtained by
(a) synthesizing double-stranded RNA using a single-stranded template, an RNA polymerase and nucleotides, wherein at least each A, each G, each C or each U residue comprises an azido group bound to its base, and at least each A, each G, each C and each U comprises an amino group bound to its base, whereby the azido and amino groups do not reside on the same type of base,
(b) removing from the resulting doublestranded RNA the single-stranded RNA containing the azido and amino group-containing nucleotides and
(c) reacting the resulting single-stranded RNA with a first modifying group which forms a bond with the azido group and a second modifying group which forms a bond with the amino group so as to obtain the single-stranded RNA,

Gold teaches that any of the RNA nucleotide bases (A, C, G or U) may be covalently modified such as to form azido adducts (para [0020]). Gold also teaches 2'-amino group modifications of bases (para [0007]). Gold does not require that the amino modifications reside on the same base as the azido modifications and the two modifications are taught independently (para [0007], [0020]). Brown teaches the synthesis of dsRNA (para [0018]) from an ssRNA template (para [0049]) utilizing a polymerase (para [0074]) and modified nucleotides (para [0025]), as well as removing and separating resulting ssRNA from dsRNA following synthesis (para [0049]). Neither Gold, Tom-Moy nor Brown teaches reacting the resulting single-stranded RNA with a first modifying group which forms a bond with the azido group and a second modifying group which forms a bond with the amino group so as to obtain the single-stranded RNA.

Seela teaches further modification of azido-derivatized nucleotides (para [0115]) and with alkynyl functional groups (para [0026], [0104]) to form aminoalkynyl adducts (para [0717]). It would have been obvious to one of skill in the art to combine the teachings of Gold, Tom-Moy, Brown and Seela to synthesize and sequence ssRNA by the claimed method because Seela's teaching of further derivatization of the amino functional group on nucleotide bases would be expected to add an additional level of uniqueness to the RNA for improved sequencing and characterization.

Claims 1-17 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.